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The Assembly, Structure and Activation of Influenza a M2 Transmembrane Domain Depends on Lipid Membrane Thickness and Composition

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The influenza A M2 protein is single-pass transmembrane protein that assembles in a tetramer, forming a pH-activated proton channel. It is essential for viral function. The tetramer of M2 transmembrane domain (M2TM), residues 22-46, is the minimal unit for proton conductance. Although substantial knowledge about M2-membrane interactions was accumulated, understanding the energy profile of the M2TM assembly and structural alterations upon channel activation in lipid membranes is incomplete.

We utilized pulse ESR spectroscopy, DEER, with spin-labeling to study the AM2TM peptide (residues 21-49) in lipid membranes. A unique cysteine residue (L46C), introduced in M2TM, was spin-labeled with MTSL. The peptide was reconstituted in membranes of pure DLPC or DOPC, DLPC:DLPS or DOPC:POPS 85:15 mol%, and DLPC:DLPS:Cholesterol or DOPC:POPS:Cholesterol 65:15:25 mol% at peptide-to-lipid molar ratio 1:500. We measure inter-spin distances and distance distributions to monitor structural alterations in M2TM residing in different lipid environment, and also upon changing the acidity from pH 8 to 5.5. Based on the modulation depth of DEER signals and the reconstructed distance distributions, our results indicate that membrane-associated M2TM exists in various oligomerization states, most likely dimers and tetramers, supposedly relevant to M2 folding. The length of lipid hydrocarbon tail and cholesterol affect M2TM oligomer assembly: The DEER modulation depth for M2TM in DLPC corresponds to dimers, whereas the increased depth measured in POPC membranes and DLPC:DLPS:Cholesterol suggests a shifted equilibrium to tetramers. The distances in DOPC membranes at both pHs were shorter (maximum at 22 Å), as expected from a closed M2 conformation. Longer distances, 27-40 Å, due to channel opening at low pH were observed clearly only in the presence of charged lipid, indicating the role of these lipids in the activation of M2 channel.

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HSP70 Associates with Phosphatidylserine Membranes via the Peptide Binding Domain

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The expression of heat shock proteins (hsp) is a natural response to an array of physiological, environmental and clinical stressors. These proteins participate in the repair and recovery from an insult and confer protection from subsequent stresses. The cytoprotective effect of hsp has been associated with their chaperone function within the cytosol. Recently, they have been found in the extracellular environment where they propagate the stress signal to avoid the dissemination of the insult. Hsp70, the major inducible form of the hsp family, does not contain any consensus secretory signal that predicts its export via the classical ER-Golgi secretory pathway. We have proposed that Hsp70 is exported by a novel mechanism that is initiated by the translocation of the protein into the plasma membrane. Furthermore, the protein is released associated with extracellular vesicles (ECV), which we speculate result in a robust activation of the immune system. We investigated the mechanism of Hsp70 insertion into membranes using liposomes. We observed that Hsp70 insertion into lipid membranes was spontaneous and specific for negatively charged lipids, such as phosphatidylserine. Using a proteomic approach, we determined that the C-terminus end of the molecule, which contains the peptide binding domain, is inserted into the lipid bilayer. The N-terminus of the molecule, containing the ATP binding site, is exposed to the external part of the liposome. These results resemble our initial studies characterizing channel activity of Hsp70 observed in artificial lipid membranes.

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Platform: Computational Methods

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Bayesian Structure Determination from Sparse Single Molecule X-Ray Diffraction Images

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X-ray free electron lasers used in single molecule experiments offer new possibilities for molecular structure determination. We propose a Bayesian method capable of extracting structure information from sparse and noisy diffraction images. We investigate two different strategies. In the first, a 'seed' model is used to determine the molecular orientation for each of the collected diffraction images, and an improved molecular transform is obtained by averaging those images in three-dimensional reciprocal space. In the second approach, a real space structure model that fits best to the entire set of diffraction images is obtained, thus enabling distinction between different structures.

We found that the achievable resolution increases with molecular mass as $m^{1/6}$, which somehow unexpectedly suggests that, at a given resolution level, structure determination is more challenging for small molecules.

As a proof of concept, we have computed the electron density for a glutathione (molecular mass 307 Da) from 20,000 synthetic diffraction images, each with 82 recorded elastically scattered photons, and up to 50% additional background noise. Alternatively, and demonstrating the feasibility of the second approach, the structure of the same molecule was also determined in a Monte Carlo refinement simulation starting from random conformations. Further, the second approach is exemplified for a ribosomal structure (molecular mass about 2.5 MDa). Our results show that it is possible to distinguish between minute structural changes associated with tRNA translocation.

Overall, our results suggest that the proposed method allows for structure determination at atomic resolution from sparse and noisy X-ray diffraction images in single molecule experiments for a broad spectrum of molecular masses.

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xMDFF: Molecular Dynamics Flexible Fitting of Low-Resolution X-Ray Structures

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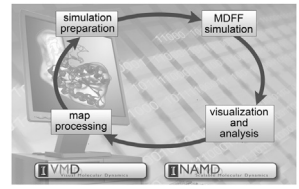
X-ray crystallography remains one of the most versatile and dominant methods for solving the all-atom structure of biomolecules. However, for relatively large systems, availability of only medium to low-resolution diffraction data often limits the determination of atomic structures. We have developed a new molecular dynamics flexible fitting (MDFF)-based approach, xMDFF, for determining structures from such low-resolution crystallographic data. xMDFF employs a real-space refinement scheme that flexibly fits atomic models into an iteratively updating electron density map. The iterations continue until the fitted structure yields R-factors lower than a predefined tolerance. xMDFF addresses significant large-scale structural deformations of the initial model to fit the low-resolution density, as has been tested with synthetic low resolution maps of D ribose binding protein. xMDFF has been successfully applied to re-refine six low-resolution protein structures of varying sizes that were already submitted to the PDB. An improvement in the R-factors is observed, and sterically and conformationally favored atomic geometries are achieved in all the six cases. Finally, via systematic refinement of a series of data form 3.6 to 7 Å, xMDFF together with electrophysiology experiments confirmed the first all-atom structure of a voltage sensing protein Ci-VSP.



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i-ATTRACT: a New Flexible Docking Approach for Investigating Protein Protein Interactions

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Many of the most important processes in the cell are carried out by large molecular machines built up from multiple proteins. However, structural data for a large fraction of known and putative complexes is still lacking. Computational docking methods aim at predicting protein complexes based on the structure of the individual constituents. A new protein-protein docking approach, i-ATTRACT has been evaluated on a large benchmark. The docking combines rigid body degrees of freedom and fully flexible interface residues in a simultaneous potential energy minimization. To our knowledge this is the first docking method performing an energy minimization in degrees of freedom of multiple scale. Procedures combining Monte Carlo sampling and energy minimization were applied as well. Refinement of rigid body docking solutions from a systematic search with unbound protein structures using ATTRACT [1] shows promising results on a large number of cases. i-ATTRACT is able to significantly improve results for initial structural deviations of up to 8 Å from bound geometries. Compared to molecular dynamics this refinement procedure comes at low computational cost but shows more efficient sampling by combining small-scale conformational rearrangements and large-scale center-of-mass displacements.